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Review article

Mechanisms governing the protective effect of 17β-estradiol and estrogen receptors against cardiomyocyte injury

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ABSTRACT

The sex hormone 17β-estradiol (E2) is the most abundant and active estrogen in premenopausal women. Studies have shown that high circulating levels of E2 are cardioprotective and are associated with reduced risk of developing heart disease in women of reproductive age. Estrogen receptors (ERs) are divided into three subtypes, namely ERα, ERβ, and GPR30, and these receptors have been shown to play important roles in E2-mediated pathways that protect cardiomyocytes from various cardiac insults, such as hypoxia, ischemic-reperfusion injury, sepsis, and hypertrophic agents. This review focuses on the role that estrogen and ER-mediated signaling pathways play in protecting cardiomyocytes against various stresses. Moreover, the therapeutic implication of selective ER-agonists on cardiomyopathy along with remaining unanswered issues are further discussed.

1. Introduction

Heart disease is a major cause of death worldwide and usually develops as a result of deteriorating myocardial function. The incidence of heart disease is low in premenopausal women but increases substantially in postmenopausal women, suggesting that sex steroid hormones protect the female heart [1]. Evidence from *in vivo* and *in vitro* studies suggests that 17 β-estradiol (E2), the most abundant and active estrogen in premenopausal women, plays a cardioprotective role by preventing cardiomyocyte apoptosis and alleviating left ventricular hypertrophy, as well as protecting against the development of cardiac fibrosis in women. Nevertheless, a few clinical trials on the effect of estrogen replacement in postmenopausal women have shown that high levels of estrogen may contribute to the development of heart disease [2,3]. Further investigations are warranted to fully understand the complex effects of estrogen and estrogen receptors (ERs) on cardiomyocyte biology prior to the clinical application of estrogen for treatment of cardiomyopathy. This article will review the current state of knowledge of estrogen signaling in cardiomyocyte protection.

2. Molecular mechanisms of ER signaling

The physiological actions of E2 are mediated by two ER subtypes, ER-alpha and ER-beta (ERα and ERβ), and ER signaling

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can be divided into genomic and nongenomic pathways. In genomic ER signaling, estrogen diffuses into cells and binds to ERs to form a nuclear estrogen–ER complex. This complex then binds to estrogen response element (ERE) sequences in the regulatory regions of estrogen-responsive genes, with consequent physiological responses. Another type of ER genomic activity occurs through protein–protein interactions with activator protein 1 (AP1) or specificity protein 1 (SP1) sites in the promoter region of estrogen-responsive genes. Both ERα and ERβ can modulate gene expression either *via* ERE-mediated signaling or by interacting directly or indirectly with other transcription factors. This mechanism represents the tight regulation of ER signaling in response to 17β-estradiol.

In nongenomic ER signaling, estrogen binds to ERs via cytoplasmic signal transduction proteins, such as mitogen-activated protein kinase, Stats (signal transducers and activators of transcription), and Src family tyrosine kinases, or through membrane-associated estrogen-binding receptors, resulting in cellular responses [4]. Binding of ERs to the p85 subunit of type I phosphoinositide-3 kinase (PI3 K), which results in increased PI3 K activity and subsequent activation of protein kinase B, governs E2- and ER-mediated pro-survival signaling in cardiomyocytes [5]. In contrast, ER signaling can be activated by growth factors regardless of the presence of E2. Growth factors such as insulin-like growth factor 1 (IGF1) and epidermal growth factor (EGF) interact with their membrane-bound receptors (receptor tyrosine kinases) and induce mitogen-activated protein kinase signaling, which in turn activates ER by changing its phosphorylation status [6].

GPR30 (G protein-coupled estrogen receptor) is a membrane-localized ER located primarily on plasma membranes, endoplasmic reticulum, and nuclear membranes [7]. This membrane-bound ER has high E2 affinity but low ligand capacity, and is considered to play an important role in rapid signaling events and rapid transcriptional activation. The role that GPR30 plays in E2-mediated cardioprotection, however, is not clear.

3. Structure of ERs

ERα and ERβ are transcribed from different genes and display distinct expression patterns as well as different ligand specificities [8]. These two ER subtypes are members of the nuclear receptor superfamily and share common structural characteristics, including five distinguishable domains, which are defined as the A/B, C, D, E, and F domains, respectively [9]. The A/B domain is thought to contribute to ER subtype-specific actions on target genes, and the two ER subtypes share less than 20% amino acid homology in this region. The domain contains activation function 1 (AF1), which provides for ligand-independent ER activation [10]. The A/B domain is associated with the development of tamoxifen resistance in some breast cancer patients. Studies have shown that excessive activation of growth factors may give rise to the possibility of ligand-independent ERα activation *via* that ligand-independent pathway [11].

The central C domain is highly conserved among two of the ER subtypes and is critical for specific DNA binding and dimerization. The D domain is the hinge domain between the DNA-binding domain and the ligand-binding domain, and is considered to play an important role in ER nuclear translocation [12,13]. The E domain is referred to as the ligand-binding domain, and ERα and ERβ share approximately 55% amino acid identity in this region. The ligand-binding domain contains a hormone-dependent activation function 2 (AF2) domain that is important for ligand binding. The F domain has less than 20% amino acid identity between the two ER subtypes, and the functions of this domain remain is unknown.

GPR30 was initially identified as an orphan G protein-coupled receptor with estrogen as its endogenous ligand. As a transmembrane ER, GPR30 activation may mediate E2 rapid cell signaling [14]. The effects of and the molecular mechanisms governing GPR30 in cardiomyocytes have not been fully evaluated.

4. E2/ER protects against *lipopolysaccharide*-induced cardiomyocyte death

Lipopolysaccharides (LPSs) are a common cause of sepsis and a common cause of sepsis-induced heart failure [15]. This outer-membrane component of Gram-negative bacteria is known to interact with toll-like receptor 4 (TLR4) on cardiomyocytes, resulting in inflammation and cardiomyocyte apoptosis [16]. Liu et al found that LPS-induced myocardial apoptosis was mediated by c-Jun N-terminal kinases 1/2. Their studies showed that JNK1/2 activated nuclear factor kappa B (NFκB), which in turn led to the release of cytochrome C as well as the overexpression and activation of pro-apoptotic proteins such as tumor necrosis factor alpha (TNFα), caspase 8, truncated BH3 interacting domain death agonis bid (t-Bid), Bcl2-associated X protein (BAX), caspase 9, and caspase 3 [17]. Elevated PI3 K-Akt activity mediated by E2 and ERα contributes to the inhibition of nuclear translocation of NFκB, and therefore diminishes LPS-induced apoptosis of cardiomyocytes (Fig. 1). This finding is consistent with that reported by Pelzer et al, who showed that E2 and ERs inhibit the nuclear localization of NFκB [18]. Their finding might explain why menopausal women with sepsis have lower mortality rates as well as a lower incidence of heart failure.

Interestingly, the ERα receptor and membrane-impermeable E2 seem to act synergistically through a similar signaling pathway to protect against LPS-induced damage in cardiomyocytes. On the other hand, previous studies have shown that selective activation of ERβ also inhibits nuclear translocation and DNA binding of NFκB in cardiomyocytes [19]. Further studies on whether ERβ can protect against LPS-induced heart disease are warranted.

5. E2/ERs protect against hypertrophic stimuli-induced cardiac hypertrophy and cardiomyocyte death

Cardiac hypertrophy is one of the most frequent causes of heart failure and can arise from a variety of cardiac insults including hypertension, excess activation of the sympathetic nervous system, or other hypertrophic agents such as angiotensin II, endothelin 1, or β-adrenergic receptor agonist

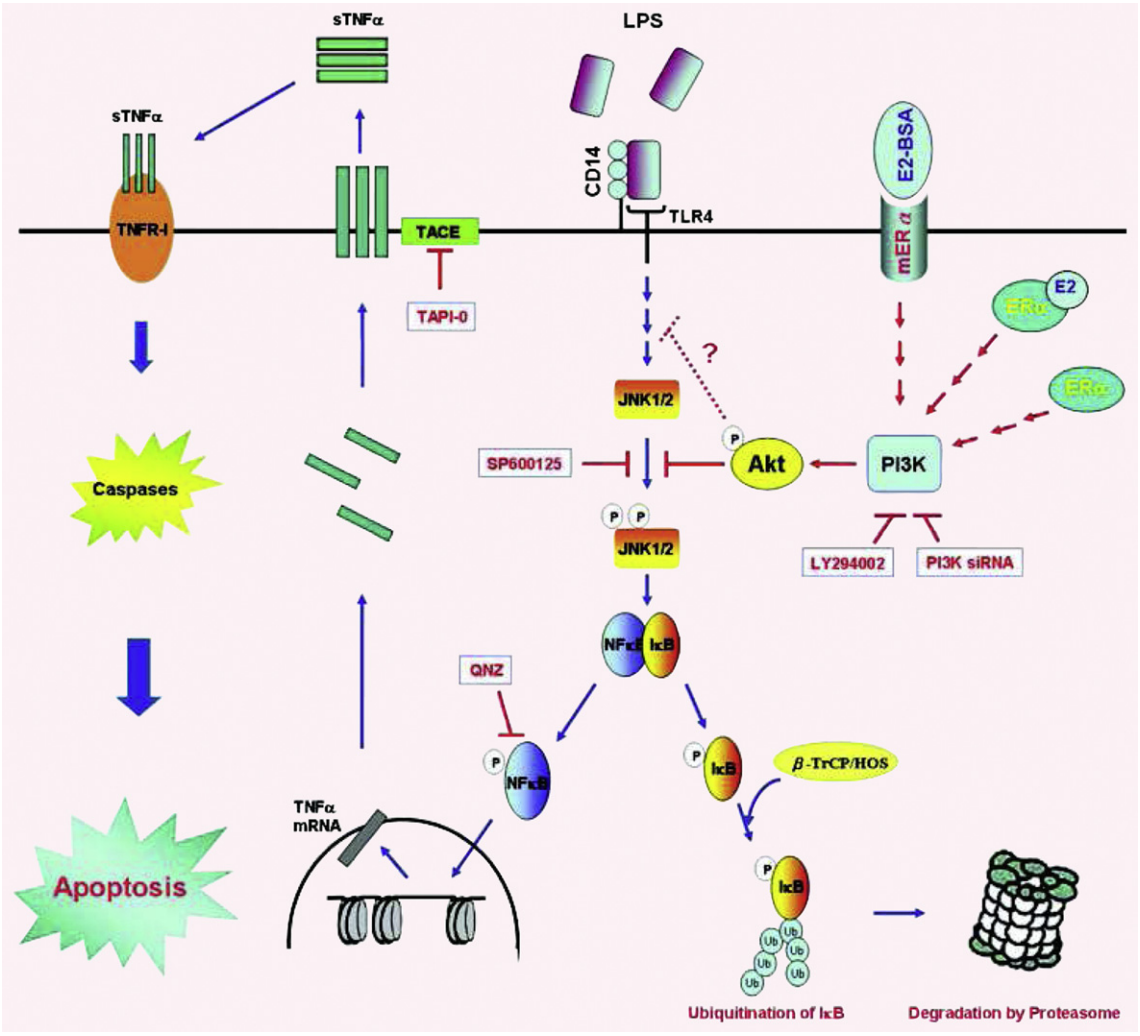

Fig. 1 – Schematic representation of how E2, BSA-E2 and ERα expression inhibit LPS-induced TNFα expression and cardiomyocyte apoptosis through activation of Akt [17]. For abbreviations, see text.

[20–22]. Cardiac hypertrophy can be characterized as an alternation in cardiac geometry (size and shape) with increases in cardiomyocyte size as well as extracellular matrix components. Pathological hypertrophy often results in cardiomyocyte apoptosis and eventually to deterioration of cardiac function.

The importance of abnormal activity of calcineurin (a calcium-sensitive phosphatase; PP2B) in cardiac hypertrophy has been investigated intensively. Calcineurin activity is increased by a variety of hypertrophic stimuli, such as angiotensin II and isoproterenol [23]. Calcineurin activation promotes the nuclear translocation of nuclear factor of activated T cells 3 (NFAT3) and activates myocyte-enhancing factor 2, resulting in the upregulation of hypertrophic genes [23,24].

The results from various animal studies indicate that estrogen may defend against the development of cardiac hypertrophy. We recently showed that E2 and ERβ alleviate isoproterenol-induced cellular calcium accumulation in cardiomyocytes by activating phospholamban (PLB) and PI3 K-Akt-murine double minute 2 (MDM2) signaling cascades [25], which increase the protein degradation of calcineurin, thereby inhibiting isoproterenol-induced myocardial cell hypertrophy and apoptosis (Fig. 2). On the other hand, ERα seems to protect against isoproterenol-induced hypertrophy and apoptosis in myocardial cells. Our laboratory has recently discovered that E2 facilitates the interaction between ERα and Src proteins in myocardial cells, and that such interactions result in the activation of the IGFIR-PI3 K-Akt and EGFR-MMP2/9-MEK1/2-ERK1/2 signaling pathways. The activated pathways mentioned above markedly decrease the levels of calcineurin-induced proapoptotic proteins and play a role in protecting cardiomyocytes from isoproterenol-induced apoptosis [26].

Interestingly, Filardo et al reported that GPR30-mediated G-protein βγ subunits (Gβγ) activation by E2 results in the activation of Src and matrix metalloproteinase-mediated cleavage of heparin-bound EGF. The latter is then able to activate the EGF receptor with subsequent acute activation of PI3 K and extracellular signal-regulated kinases (ERK) [27].

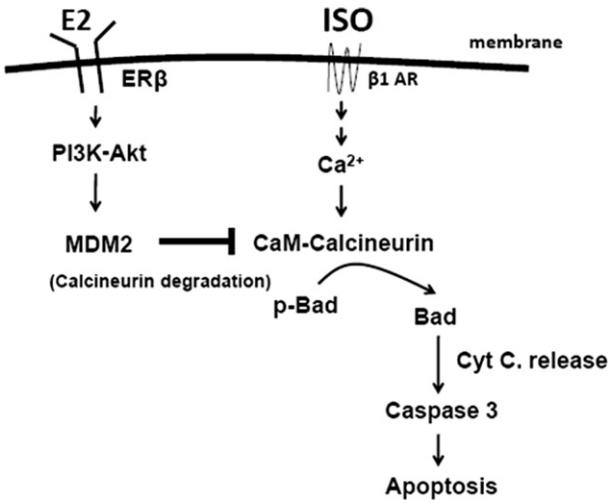

Fig. 2 – Schematics of hypothetical model of E2/ERβ-enhanced calcineurin protein degradation by PI3K /Akt/ MDM2 signaling activation, contributing to inhibition of ISO-induced myocardial cell apoptosis. [25]; Bad = Bcl-2-associated death promoter protein; Cyt C. = cytochrome C; ISO = isoproterenol. For abbreviations, see text.

This pathway is very similar to what we have observed in E2- and bovine serum albumin (BSA)-E2-treated cardiomyocytes as well as in tetracycline-inducible gene expression system (Tet-on) cardiomyoblast cells that overexpress ERα, suggesting that the EGFR-MMP2/9-MEK1/2-ERK1/2 pathway might be the crucial pro-survival signal for E2 and ERs in cardiomyocytes in response to hypertrophic insults. These data raise an interesting question concerning the cardioprotective effects of GPR30 against hypertrophic insults.

In addition, we have also identified a novel ERα-mediated cardioprotection mechanism (Fig. 3). Under isoproterenol stimulation, activated ERα interacts with glycogen synthase kinase 3 beta (GSK3β) in cardiomyocytes, with subsequent upregulation of I2PP2A (a potent inhibitor of protein phosphatase [PP] 2A). This prevents PP1 activation by PP2A and contributes to the stabilization of intracellular calcium concentration by suppressing the association between PP2A and NCX (a cardiac Na^{+}/Ca^{2+} exchanger), the consequent activation of isoproterenol-induced calcineurin, as well as apoptosis [28].

6. E2/ERs protect against myocardial oxidative stress and ischemic-reperfusion injury

Ischemic heart disease (IHD) is the most common cause of death and hospitalization in many Western countries [29]. Reduced blood supply induces a hypoxic situation in heart muscle, which usually stimulates the production of cytokines, such as interleukin 6, and subsequently leads to myocardial inflammation and cardiomyocyte apoptosis [30,31]. In addition, re-establishment of blood flow in infarcted myocardial tissue can paradoxically cause further damage to ischemic tissue, a condition referred to as 'ischemia-reperfusion injury'. Epidemiological studies indicate that premenopausal women are at a lower risk of developing IHD than men of similar age [32]. Furthermore, favorable effects of estrogen replacement therapy, including smaller infarct size and reduced apoptosis in the peri-infarct zone of the left ventricle, have been shown in a few animal models of ischemia-reperfusion injury [33]. These preclinical as well as epidemiological studies suggest that estrogen has a cardioprotective effect against myocardial ischemia-reperfusion injury.

Reactive oxygen species (ROS) are the primary cause of cardiomyocyte death during the reperfusion stage of ischemia-reperfusion injury [34,35]. Attenuation of ROS generation as well as the increased activity of the GSH/GRX (γ-glutamylcyteinyl glycine/glutaredoxin) system is thought to play an important role in E2- and ER-mediated cardioprotection against ischemia-reperfusion injury. Liu et al suggested that E2 activation of ERα or ERβ results in the activation of PI3 K with subsequent inhibition of ischemia-reperfusion injury-induced ROS [36]. This ER-mediated protection can be summarized as follows: First, activated PI3 K increases p38β activity and downregulates the activity of p38α, leading to inhibition of p53 activation with subsequent ischemia-reperfusion-induced cardiomyocyte apoptosis. Second, increased levels of S-nitrosylation proteins by activation of endothelial nitric oxide synthase (eNOS) results in decreased oxidative stress and ROS generation [37]. In addition, E2 also mediates cardioprotection either through ERα or ERβ genomic pathways. Activation of either ERα or ERβ upregulates the expression of gamma-glutamylcystenine synthetase (γ-GCS) and glutathione S-transferase (GSH) and then actives the GSH/GRX system, resulting in reduction of the oxidative state of Akt; this thereby inhibits PP2A activation and protects cardimyocytes from oxidative stress-induced apoptosis by preserving Akt activity. Meanwhile, ERα interacts with AP1 and SP1 on γ-GCS and GSH genes, and ERβ binds to an EpRE-like 1 element, a possible novel kind of ERE, resulting in the upregulation of γ-GCS and GRX, and thereby preventing myocardial cell apoptosis under oxidative stress [38].

GPR30 protects against myocardial ischemia-reperfusion injury through activation of the PI3 K/Akt pathway [39]. Consequently, we conclude that all three ER subtypes contribute to E2-mediated cardioprotective effects against ischemia-reperfusion injury through activation of the PI3 K-Akt nongenomic pathway, which leads to the activation of NOS/nitric oxide signaling in cardiomyocytes.

In addition to ROS, hypoxia-induced BNIP3, a Bcl-2 family of pro-apoptotic proteins comprising a subclass of SH3-only proteins, plays an important role in the development of hypoxia-induced cardiac hypertrophy and cardiomyocyte death [40]. We have recently found that E2 protects against BNIP3-induced apoptosis and that the protective role E2 plays might be governed by genomic and nongenomic effects. Our preliminary data suggest that ERα may bind to the regulatory region of the BNIP3 gene, which is probably located on the AP1 or NFκB binding site within the promoter, leading to the suppression of BNIP3 transcription levels. Meanwhile, the binding of ERα to BNIP3 seems to facilitate the proteasome-mediated degradation of BNIP3. These two

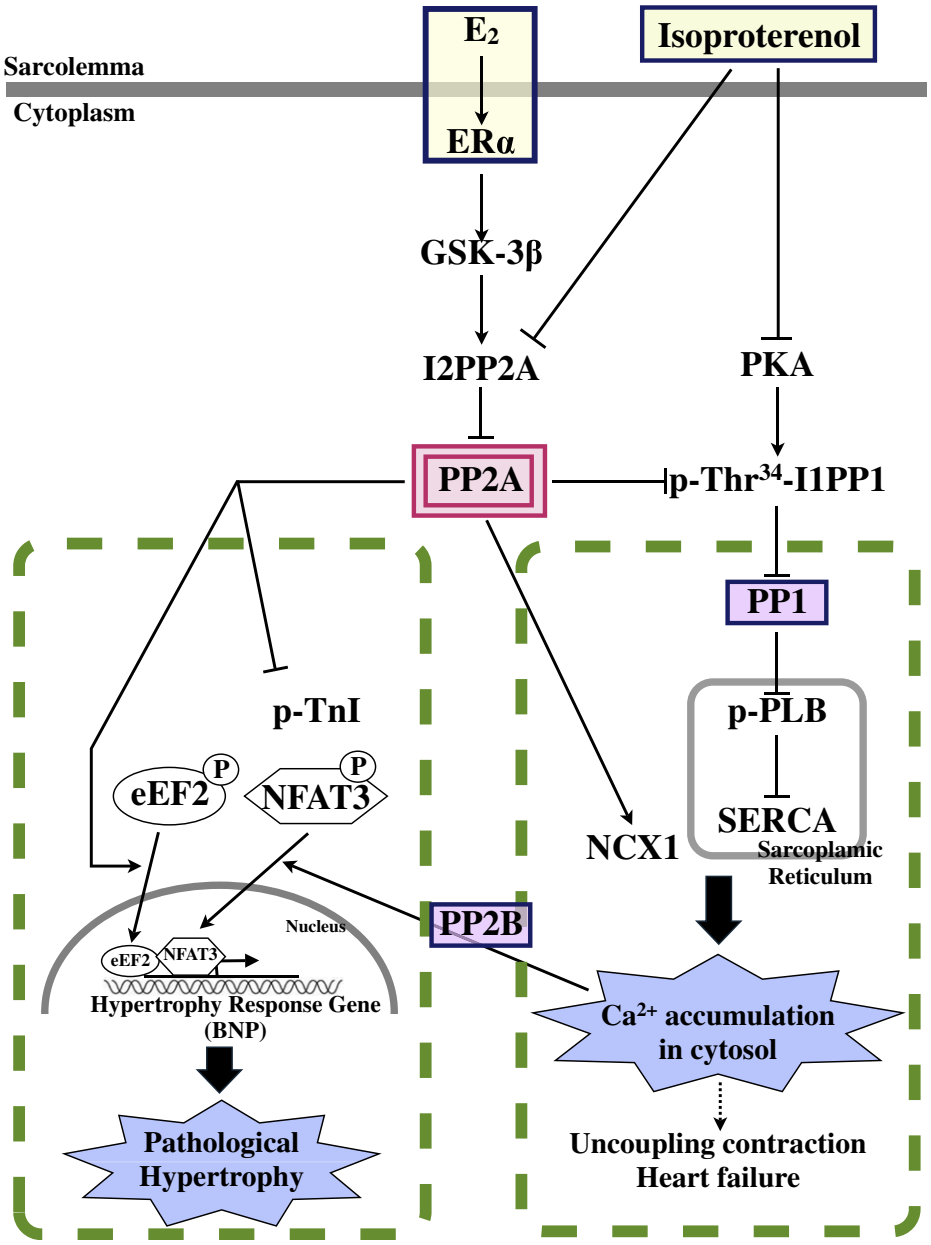

Fig. 3 – Schematics of hypothetical model of E2 and ERα inhibiting myocardial cell hypertrophy by preventing cytosolic calcium accumulation through GSK-3β & I2PP2A activation, then inhibiting PP2A to activate PP1 and suppress the association between PP2A and NCX. [28]; PKA = Protein kinase A. For abbreviations, see text.

distinct ERα-meditated effects contribute to the reduction of BNIP3-induced cardiomyocyte apoptosis [41].

In addition, we have further explored the possible role of macroautophagy (hereafter referred as autophagy) in BNIP3-induced apoptosis and investigated the possible influence of estrogen and ERs on autophagy. Our data suggest that over-expression of ERα in cardiomyocytes can mitigate BNIP3-induced autophagy and apoptosis. However, we found that necrosis marker cTnT was significantly increased when autophagy was inhibited by 3-methyladenine. Further investigations are needed to clarify the effects of estrogen and its receptors on autophagy in cardiomyocytes.

7. E2/ERs protect against IGF2 receptor death signals in cardiomyocytes

The IGF 2 receptor (IGF2R), also called the cation-independent mannose-6-phosphate receptor, is a protein that in humans is encoded by the *IGF2R* gene. IGF2R is a multifunctional protein receptor that binds IGF2 at the cell surface and mannose-6-phosphate-tagged proteins in the trans-Golgi network [42]. Although IGF2R has been shown to clear IGF2 to attenuate signaling, the function of IGF2R in heart tissue is poorly understood.

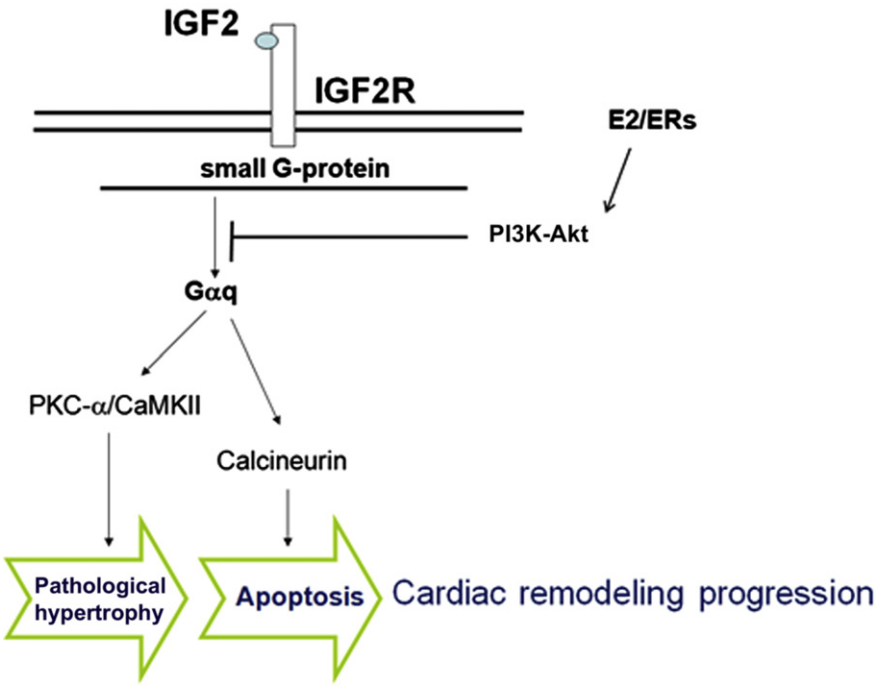

Fig. 4 – A hypothetical working model for E2/ERs against the IGF2R signaling pathway induces the myocardium cell hypertrophy and apoptosis [46]. For abbreviations, see text.

Our previous studies found that activation of IGF2R in cardiomyocytes induced by hypertension, angiotensin II, and inomycin, as well as overexpression of IGF2R, not only leads to cardiomyocyte apoptosis through the Gαq–calcineurin pathway, but also contributes to MMP2/9 (matrix metalloproteinase) activation and myocardial extracellular matrix (ECM) remodeling [43–45]. These findings suggest that the suppression of IGF2R signaling pathways may be a good strategy to prevent the progression of pathological hypertrophy. Recently, we investigated the effects of E2 on IGF2R-activated myocardial cells and found that the activation of the PI3 K-Akt pathway by E2 markedly attenuated IGF2R-induced apoptosis and hypertrophy in cardiomyocytes [46]. These data indicate that E2 has protective effects against IGF2R-induced hypertrophy and cardiomyocyte death (Fig. 4). More research is necessary to characterize whether this protection is ER-dependent and to determine the precise mechanisms responsible for this cardioprotection.

8. Conclusions and future perspectives

ERα, ERβ, and GPR30 confer cardioprotective effects against various stresses by preventing myocardial cell apoptosis and cardiac hypertrophy. Our laboratory is investigating the possible regulation of autophagy by estrogen and ER-mediated cardioprotection. This might be the critical step to fully reveal the complex cellular mechanisms of estrogen and ERs in cardiomyocytes, owing to the fact that the alternation of cardiac basal autophagy (either an increase or a decrease) is demonstrably involved in various heart diseases, such as ischemic injury, cardiac hypertrophy, cardiac remodeling, and heart failure.

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